Report No. IITRI-L6023-9
(Quarterly Status Report)

LIFE IN EXTRATERRESTRIAL ENVIRONMENTS

Contract No. NASr-22

National Aeronautics and
Space Administration
Washington, D.C.

IIT RESEARCH INSTITUTE

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February 15 to May 31, 1967

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I. INTRODUCTION

Soil ecology experiments studying the growth responses of microorganisms in different soils were completed. The initial studies dealt with the factors related to the probability of survival and growth of an organism in soil which, in turn, is related to the probability of extraterrestrial contamination. Bacillus cereus, Lactobacillus plantarum, Pseudomonas aeruginosa, Putrefactive Anaerobe (PA 3679), Staphylococcus aureus, and Streptomyces albus were inoculated into brunizemic (prairie), desertic, and podzolic (tree) soils. Additional factors studied were available moisture (water availability, a_w) and a constant or daily fluctuating freeze-thaw incubation temperature.

The daily freeze-thaw (DFT) cycles elicited four general types of growth response from the bacteria: (1) Enhanced growth or survival with DFT cycles; (2) No enhancement of growth or survival with DFT cycles; (3) A growth lag produced by DFT cycles, and (4) A suppression of growth with DFT cycles.

The soil types provided substrates with different pH's, organic content, soluble cations, and clay content. The podzolic soil had a pH of 5.7, 1.15% organic carbon, a cation exchange capacity (CEC) of 10.30 me/100 g of soil, and 13.5% clay. The brunizemic soil had a pH of 6.8, 3.73% organic carbon, a CEC of 23.81 me/100 g of soil, and 27.6% clay. The desert soil had a pH of 8.4, 0.27% organic carbon, a CEC of 27.24 me/100 g of soil, and 48.8% clay.

The brunizemic soil provided the best substrate for growth and survival in the test environments of the selected bacterial species. B. cereus, L. plantarum, P. aeruginosa, S. aureus, and S. albus grew in the brunizemic soil; L. plantarum, S. aureus, and S. albus grew in the podzolic soil, while B. cereus, PA 3679, and S. albus were the only organisms to survive in the desert soil.

Population maxima from growth in brunizemic and podzolic soils were 3 to 6 logs higher than initial numbers of viable cells which were as low as 100/g of soil. The 100 cells/g of soil was not a limiting value but the lowest concentration tested.

II. EXPERIMENTAL PROCEDURES

Stock culture preparation of B. cereus, P. aeruginosa, PA 3679, and S. aureus were described in Report No. IITRI-L6023-5; L. plantarum in Report No. IITRI-L6023-6; and S. albus

in Report No. IITRI-L6023-7. All stock culture cell suspensions were stored at 4°C until used. B. cereus and PA 3679 spore suspensions were heat-shocked at 80°C for 10 min just before use.

All tubes contained 1 g of previously sterilized soil, sufficient water to establish the desired a_w, and an Earth atmosphere at standard pressure (approximately 1013 mb). The tubes were inoculated with a single species of organism at predetermined cell populations and sealed. Half the tubes were incubated at 35°C, and half received a diurnal freeze-thaw cycle with an 8-hr freeze. Tubes were sampled immediately and at 7, 28, and 56 days.

Bacterial counts are reported as averaged counts of two plates from each of three tubes. Incubation was at 35, 30, or 25°C for 1 to 5 days, depending on the bacterial species.

The minimum a_w requirements of the different organisms were established with the method of Scott (1953. Austral. J. Biol. Sci. 6, 549-64) using a mixture of triple salts, NaCl:KCl: Na_2SO_4, in a molal ratio of 5:3:2 with trypticase soy broth (BBL). Table 1 shows the different molal concentrations of the triple salts used and the resultant a_w levels.

Table 1

MOLAL CONCENTRATIONS OF SALTS REQUIRED TO ADJUST TRYPTICASE SOY BROTH (BBL) TO VARIOUS VALUES OF a_w AT 25°C

Final a_w in Medium	NaCl (m)	KCl (m)	Na_2SO_4 (m)
0.99	-	-	-
0.96	0.5805	0.3483	0.2322
0.94	0.869	0.521	0.348
0.92	1.149	0.690	0.460
0.90	1.418	0.851	0.567
0.88	1.663	0.998	0.665
0.86	1.921	1.153	0.768

III. RESULTS AND DISCUSSION

A. Soil Characteristics

The chemical and physical properties of the three soils used in this study are given in Table 2.

Exchangeable cations were determined by reacting soil with ammonium acetate, leaching with about 200 ml 1 N HCl, and determining amount of ammonium nitrogen released by Kjeldahl method. The Walkley-Black Method was used to determine organic carbon.

Table 2

CHEMICAL AND PHYSICAL PROPERTIES OF DESERT, BRUNIZEMIC, AND PODZOLIC SOILS

Classification	Entisol (desert)	Mollisol (brunizemic)	Alfisol (podzolic)
Collection sites	Riverside County, California	Kane County, Ill.	Kane County, Ill.
Elevation, ft. above sealevel	45	680	930
Drainage	Good	Good	Good
Exchangeable cations, me/100 g of soil			
Ca	69.88	16.06	5.40
Mg	1.30	6.24	2.47
K	0.15	1.05	0.13
Na	106.25	0.08	0.05
Cation exchange capacity, me/100 g of soil	27.24	23.81	10.30
Soluble salt conductivity, mmhols/cm	100.0	1.3	0.7
Base saturation, %	651.9	98.4	78.4
pH, 1:1 ratio	8.4	6.8	5.7
Organic carbon, %	0.27	3.73	1.15
Moisture, %			
1/3 atm (Field Cap.)	34.47	25.59	16.91
15 atm (Perm. Wilt)	17.89	15.68	6.57
Particle size distribution, %			
Sand (2-.050 mm)	35.6	17.7	14.6
Silt (.050-.002 mm)	15.6	54.7	71.5
Clay (.002-.0002 mm)	48.8	27.6	13.5
Textural classification	Clay	Silt loam	Silt loam

Water sorption isotherms for the three soil types are given in Figure 1. Each data point on a curve represents an average of 6% moisture and 11 a_w determinations. It was shown that less water was required to establish a particular a_w value with the podzolic soil than with either the brunizemic or desert soils. This same fact is apparent from the field capacity (FC) and permanent wilt (PW) moisture percentages in Table 2 which shows the podzolic soil having a lower FC and PW percent than the other two soils. A soil's ability to bind water effectively is dependent upon several factors: ionic concentration, organic content, structure, and, as with the desert soil, clay content.

Extrapolation of data from the isotherms for the brunizemic and podzolic soils indicated that the maximum amount of water added produced a_w values in the 0.98 to 0.99 range. Similar a_w values were not reached with the desert soil and was the result of the high clay content and cation concentration.

B. Minimum a_w Requirements

The minimum a_w requirements of the different organisms are shown in Table 3.

Minimum a_w requirements for S. albus were determined with the concentrated medium method (Scott, J. W. 1953, Austral. J. Biol. Sci. 6, 549-564) and a 0.96 a_w was selected.

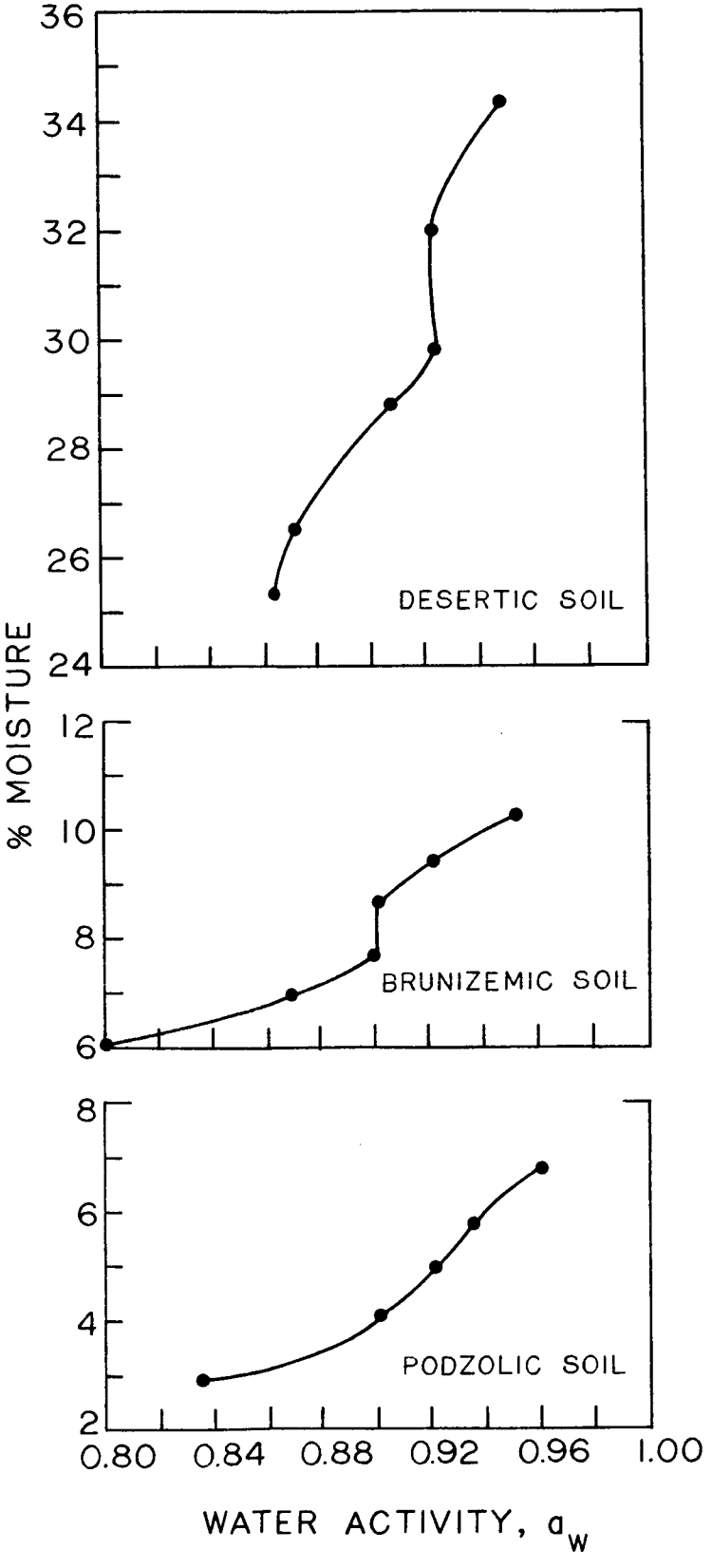

FIG. 1 WATER SORPTION ISOTHERMS OF DESERTIC, BRUNIZEMIC, AND PODZOLIC SOILS.

Table 3

MINIMUM a_w REQUIREMENTS OF SELECTED BACTERIAL SPECIES BY THE TRIPLE SALTS METHOD

Organism	a_w .99	.96	.94	.92	.90	.88	.86
B. cereus	1[a]	1	1	2[b]	-	-	-
PA 3679	1	7[b]	28	-	-	-	-
L. plantarum	2	7[b]	-	-	-	-	-
P. aeruginosa	1	3[b]	-	-	-	-	-
S. aureus	1	2	2	2	3	14[b]	-

[a]Designates days of incubation after which visible growth was present in test tubes.

[b]Minimum a_w selected for these studies.

C. Growth Response of Bacteria in Brunizemic Soil

The growth responses of six microorganisms in brunizemic soil are shown in Figures 2-7.

1. Maximum a_w

The daily freeze-thaw (DFT) cycle produced four general types of growth response with the bacteria studied:

(1) The DFT enhanced growth or survival.

(2) No enhancement of growth or survival from the DFT cycle.

(3) The DFT cycle produced a growth lag.

(4) The DFT suppressed growth.

Enhanced growth and survival with a DFT cycle occurred at the maximum a_w concentration with S. aureus and L. plantarum, Figures 2 and 3. S. aureus growth was more rapid and higher population maxima were reached with a DFT cycle than with constant temperature (CT), Figure 2. This may be caused by a greater availability of nutrients since freezing of soil does increase the concentration of water extractable amino acids and sugars (Ivarson, K. C. and F. J. Sowden, 1966. Can. J. Soil Sci. 46, 115-120; Ivarson, K. C. and U. C. Gupta, 1967. Can. J. Soil Sci. 47, 74-75). Growth of L. plantarum with both CT and DFT cycle was similar, Figure 3. The series at CT had a faster die-off than the DFT cycle series.

P. aeruginosa and B. cereus growth responses were the same with both temperature conditions with one outstanding exception, Figures 4 and 5. With a DFT cycle P. aeruginosa established itself with an inoculum of approximately 5×10^2 cells/g of soil but was not established with an inoculum of approximately 10^2 cells/g, Figure 4.

The growth of S. albus was better with CT than DFT cycle, Figure 6. The DFT cycle limited population maxima 2 to 3 logs lower than the CT series and could, in part, be related to the growth kinetics of this organism. That is, the generation time of S. albus may approach the duration time of the thaw portion of the DFT cycle or the death rate approximates the generation time. Both factors could produce a stationary population.

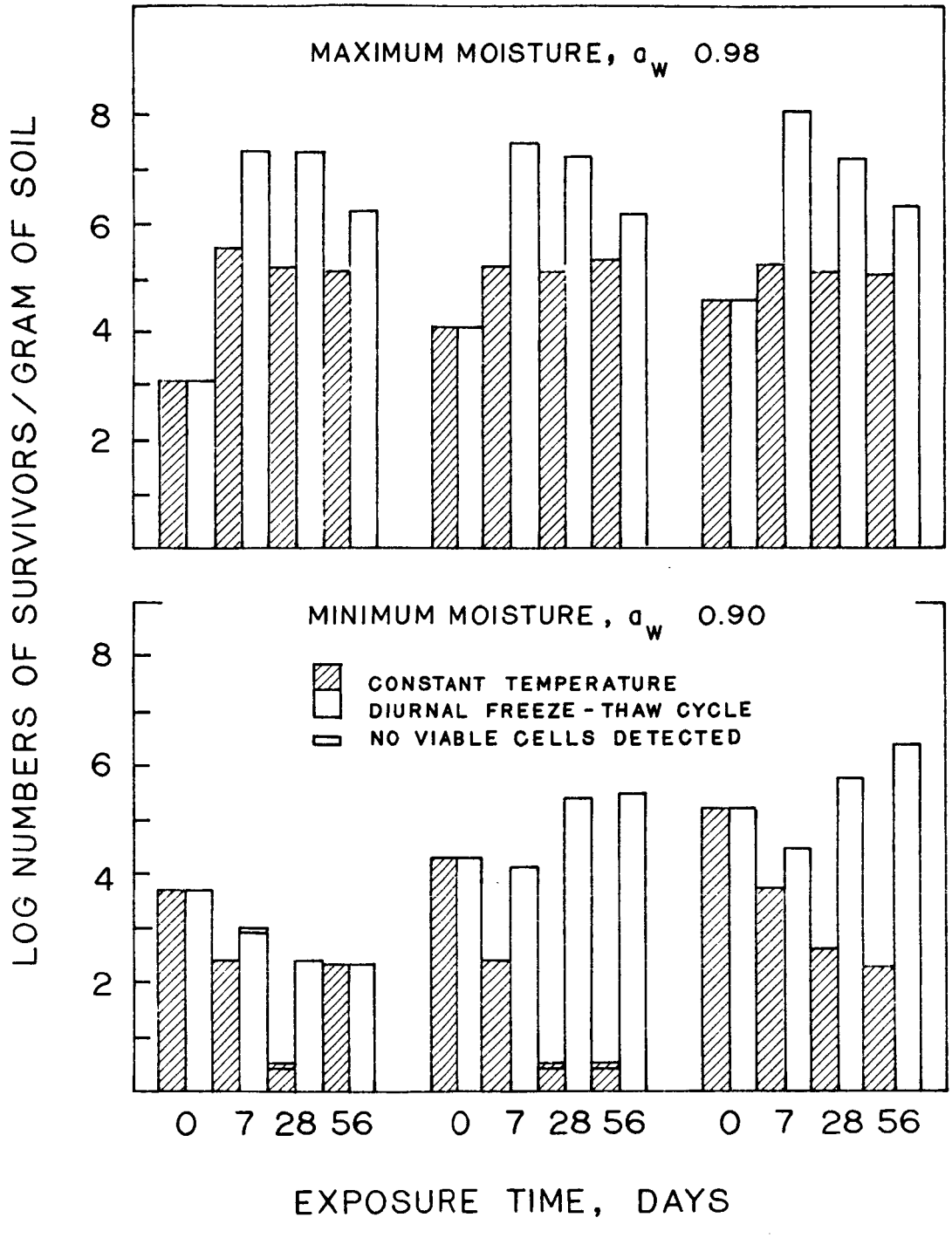

FIG. 2 RELATIONSHIP OF MOISTURE, TEMPERATURE, AND INITIAL CELL NUMBERS OF STAPHYLOCOCCUS AUREUS IN BRUNIZEMIC SOIL.

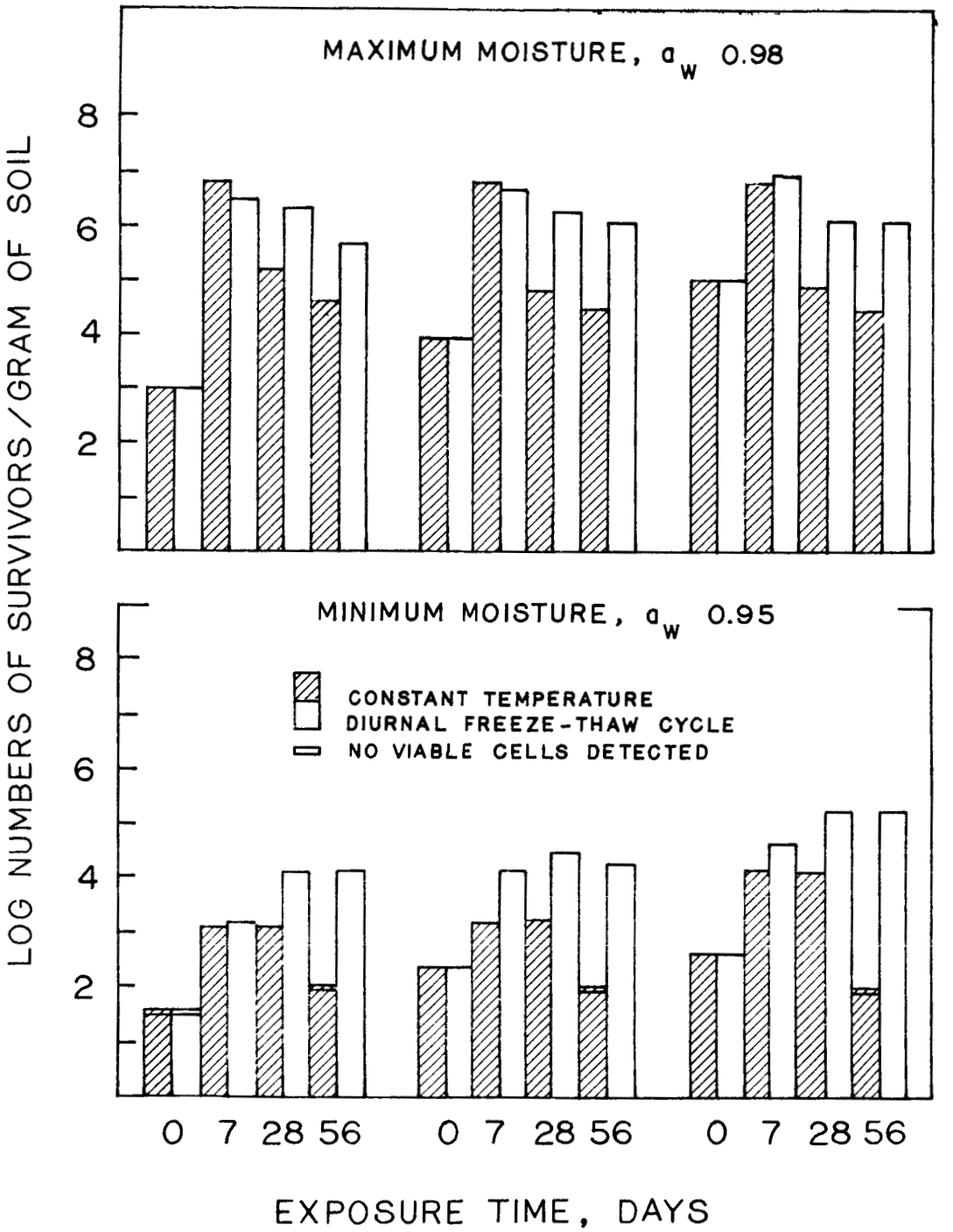

FIG. 3 RELATIONSHIP OF MOISTURE, TEMPERATURE, AND INITIAL CELL NUMBERS OF LACTOBACILLUS PLANTARUM IN BRUNIZEMIC SOIL.

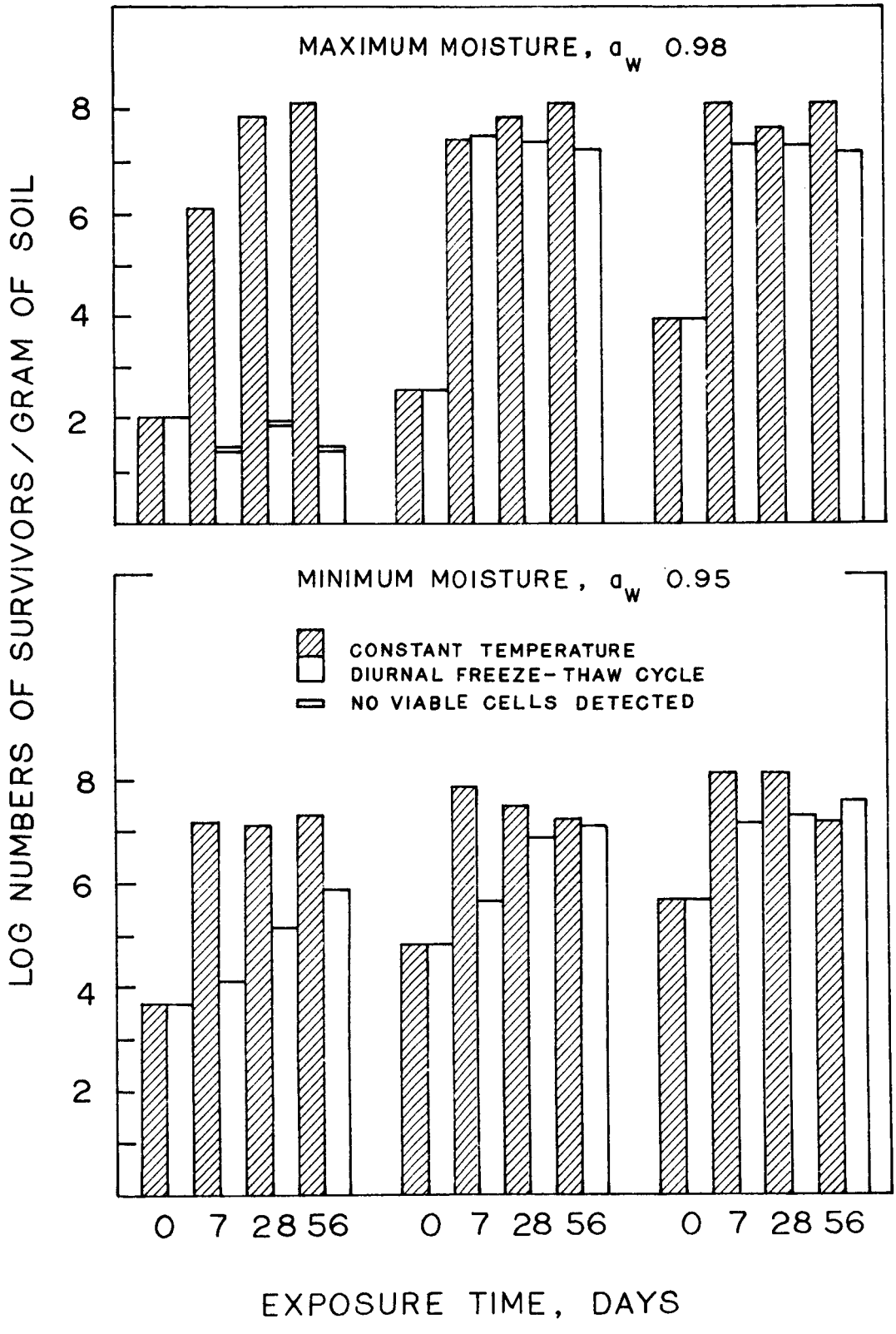

FIG. 4 RELATIONSHIP OF MOISTURE, TEMPERATURE, AND INITIAL CELL NUMBERS OF PSEUDOMONAS AERUGINOSA IN BRUNIZEMIC SOIL.

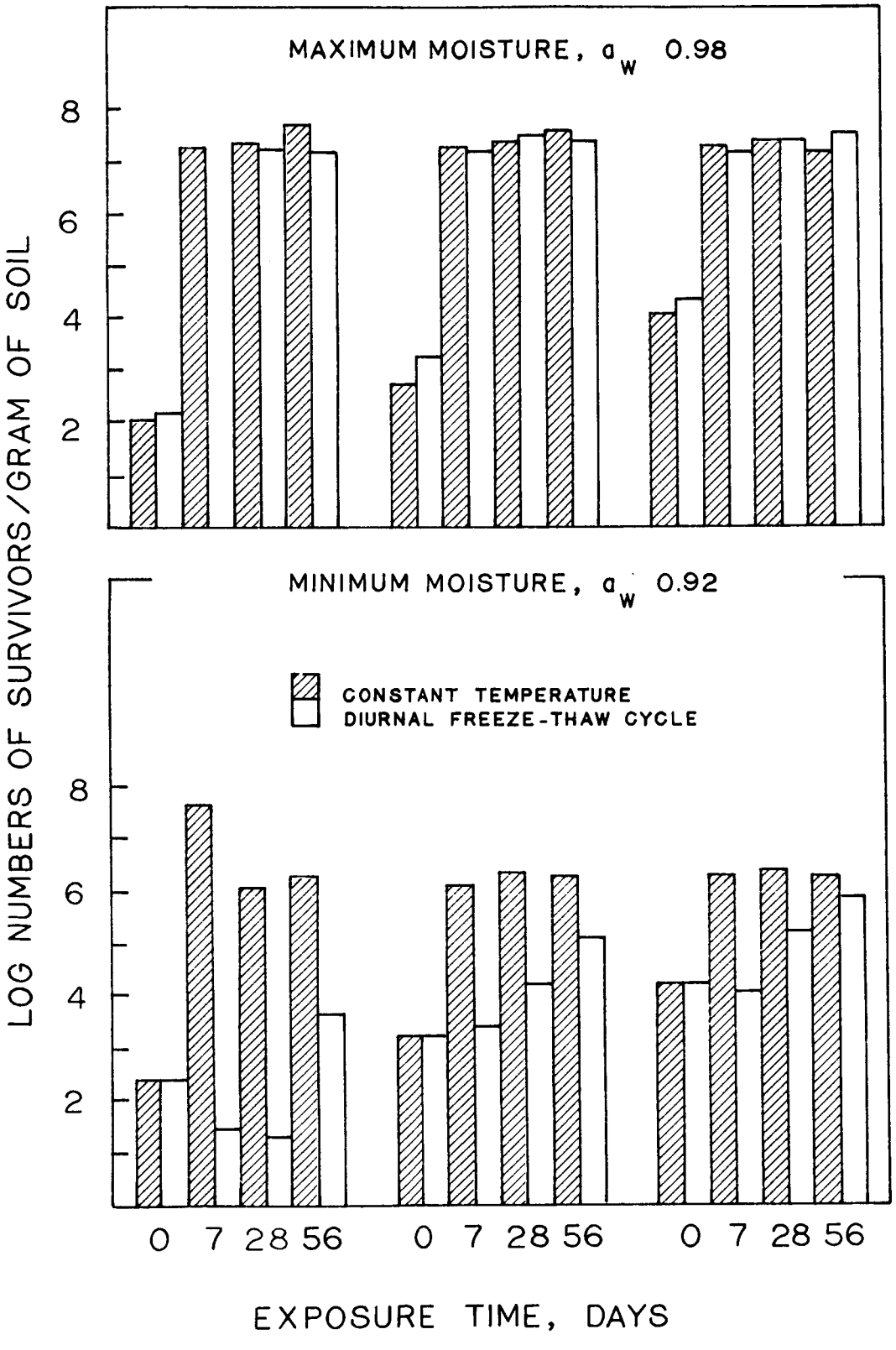

FIG. 5 RELATIONSHIP OF MOISTURE, TEMPERATURE, AND INITIAL CELL NUMBERS OF BACILLUS CEREUS IN BRUNIZEMIC SOIL.

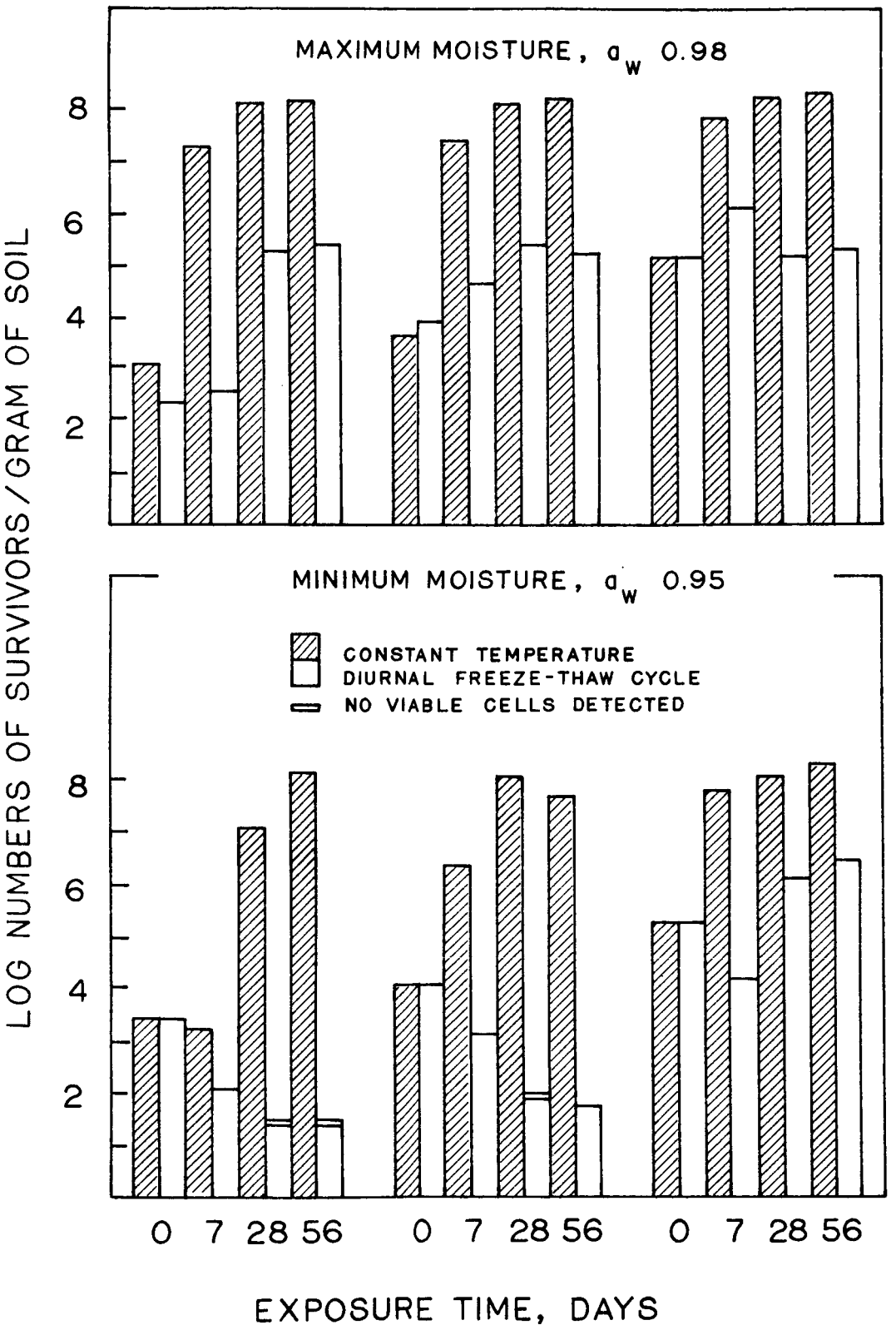

FIG. 6 RELATIONSHIP OF MOISTURE, TEMPERATURE, AND INITIAL CELL NUMBERS OF STREPTOMYCES ALBUS IN BRUNIZEMIC SOIL.

PA 3679 did not grow and there were no survivors after 56 days, Figure 7. The presence of oxygen was perhaps the major factor for lack of growth. The tubes in these experiments were intentionally not flushed with nitrogen/carbon dioxide gas mixture to determine whether or not the soil would provide protection.

2. Minimum a_w

The minimum a_w levels generally limited population maxima 1 to 3 logs lower than maximum a_w for the different bacterial species. The same general types of growth responses present with maximum a_w were also noticed with minimum a_w's. The DFT cycle enhanced the growth and survival of both S. aureus and L. plantarum, Figures 2 and 3; delayed the growth of P. aeruginosa and B. cereus, Figures 4 and 5; and suppressed the growth of S. albus, Figure 6.

In the DFT cycle series there was an effect of inoculum concentration on growth of S. albus, Figure 6. The viable cell count increased over 56 days with an inoculum of 10^5 cells/g of soil but decreased with inocula of 10^3 and 10^4 cells/g of soil.

PA 3679 showed a progressive decrease in viable cell count over the 56 day period because of aerobic conditions, Figure 7.

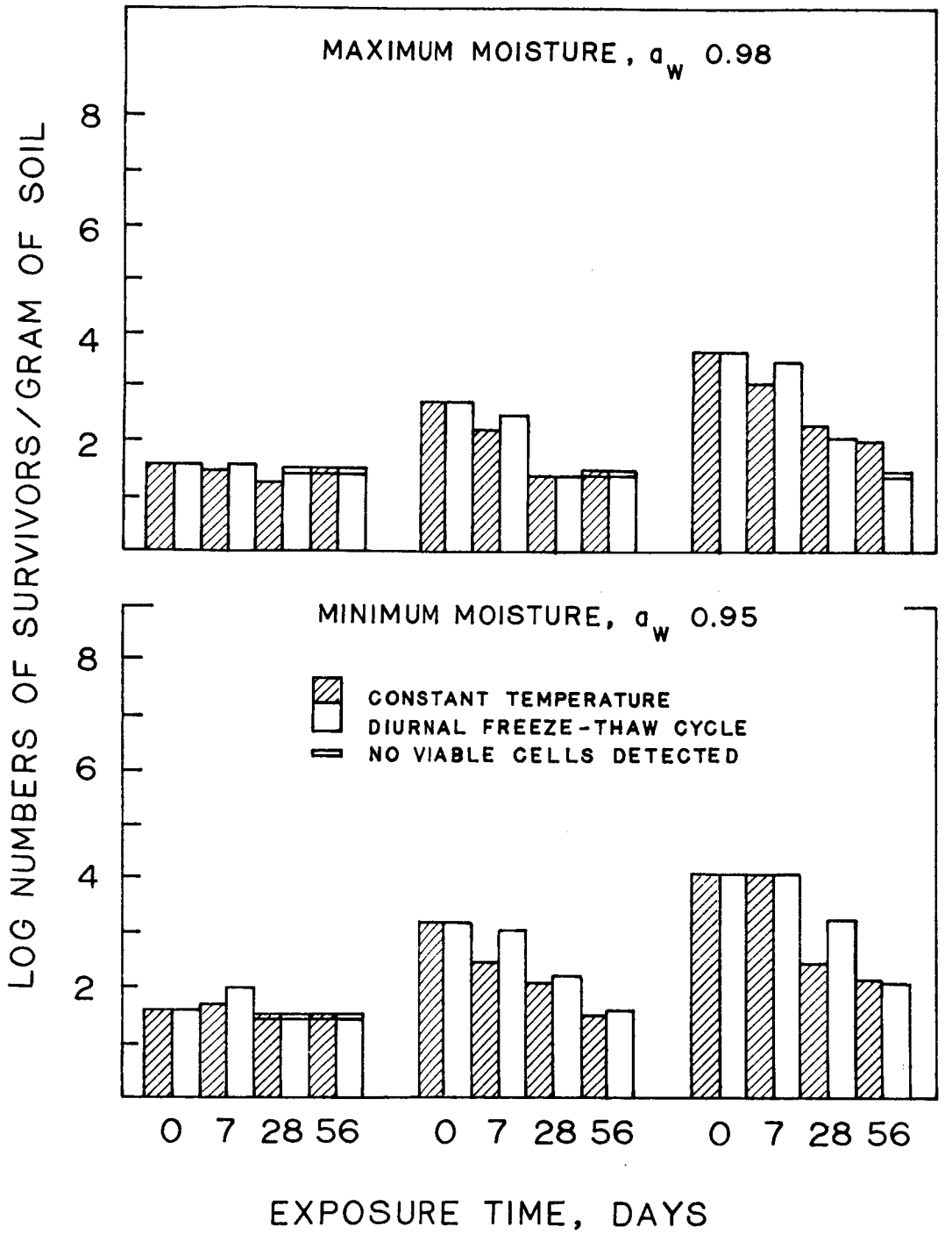

FIG. 7 RELATIONSHIP OF MOISTURE, TEMPERATURE, AND INITIAL CELL NUMBERS OF PUTREFACTIVE ANAEROBE 3679 IN BRUNIZEMIC SOIL.

D. Growth Response of Bacteria in Podzolic Soil

Figures 8 to 13 show the growth responses of these same organisms to podzolic soil. In general, the growth responses of the organisms were 1 log or more lower in podzolic soil than in brunizemic soil. There are several reasons for this lowered response:

(1) Lower cation exchange capacity (CEC) of podzolic soil.

(2) Lower pH of podzolic soil.

(3) Lower organic content of podzolic soil.

The CEC of podzolic soil was 10.30 me/100 g of soil compared to 23.81 me/100 g for brunizemic soil. Studies with soil containing clay have shown a direct relationship between growth of various soil microorganisms and the CEC of the soil (Stotzky, G. and L. T. Rem, 1966. Can. J. Microbiol. 12, 547-563; Stotzky, G., 1966. Can. J. Microbiol. 12, 831-848; and Stotzky, G., 1966, Can. J. Microbiol. 12, 1235-1246). Increased CEC of soil enhanced buffering capacity and allowed better growth of pH sensitive microorganisms.

The low pH of 5.7 of the podzolic soil compared to 6.8 of the brunizemic soil also effected growth of bacteria. Although there are numerous examples of microorganisms tolerating high (basic) or low (acid) pH extremes, a pH range from 6.0 to 7.5 could be considered as optimal. Substrates with pH's outside this range generally inhibit or limit bacterial growth.

The third, and equally important, factor limiting bacterial growth in the podzolic soil was the low organic content of 1.15% compared to 3.73% for the brunizemic soil.

The same type growth responses reported for the brunizemic soil were also noticed with the podzolic soil.

1. Maximum a_w

Enhanced growth and survival of L. plantarum and S. aureus occurred with a DFT cycle, Figures 8 and 9. The DFT cycle delayed and suppressed growth of S. albus, Figure 10 and B. cereus did not grow, Figure 11.

P. aeruginosa and PA 3679 did not survive in the podzolic soil after 56 days with a DFT cycle, Figures 12 and 13. The effect of inoculum concentration, similar to brunizemic soil, occurred with P. aeruginosa in the podzolic soil, Figure 12. Inoculum of approximately 5×10^2 cells/g of soil were not sufficient for the organism to establish itself with a DFT cycle; and 10^3, but not 10^2, cells/g were required for growth in the CT series.

The growth response of PA 3679 was poor. No survivors were recovered from either the CT or DFT cycle series after 56 days.

2. Minimum a_w

The DFT cycle enhanced growth and survival of L. plantarum, Figure 8, although population maxima were 1 to 1.5 logs lower than with maximum a_w. With minimum a_w growth of L. plantarum

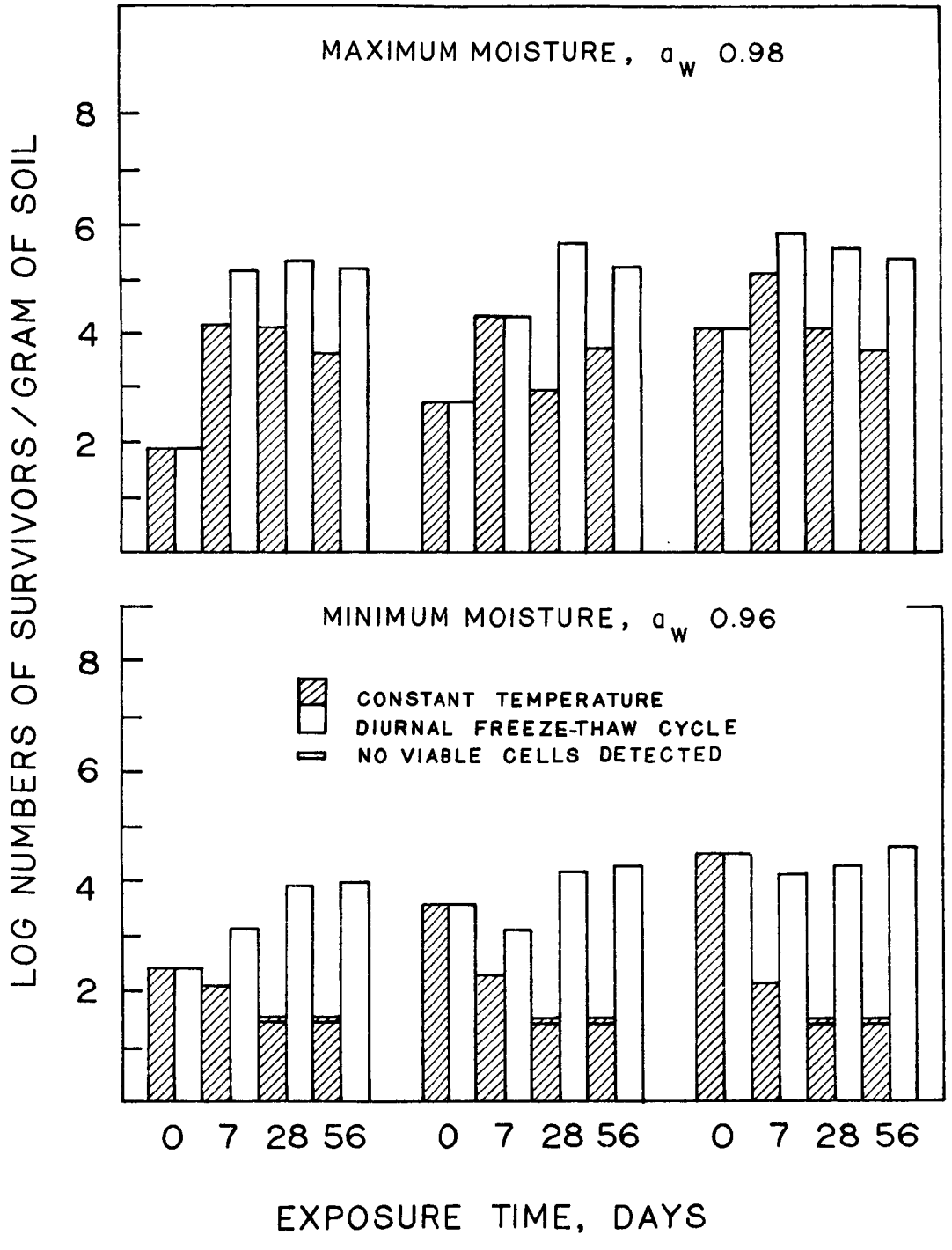

FIG. 8 RELATIONSHIP OF MOISTURE, TEMPERATURE, AND INITIAL CELL NUMBERS OF LACTOBACILLUS PLANTARUM IN PODZOLIC SOIL.

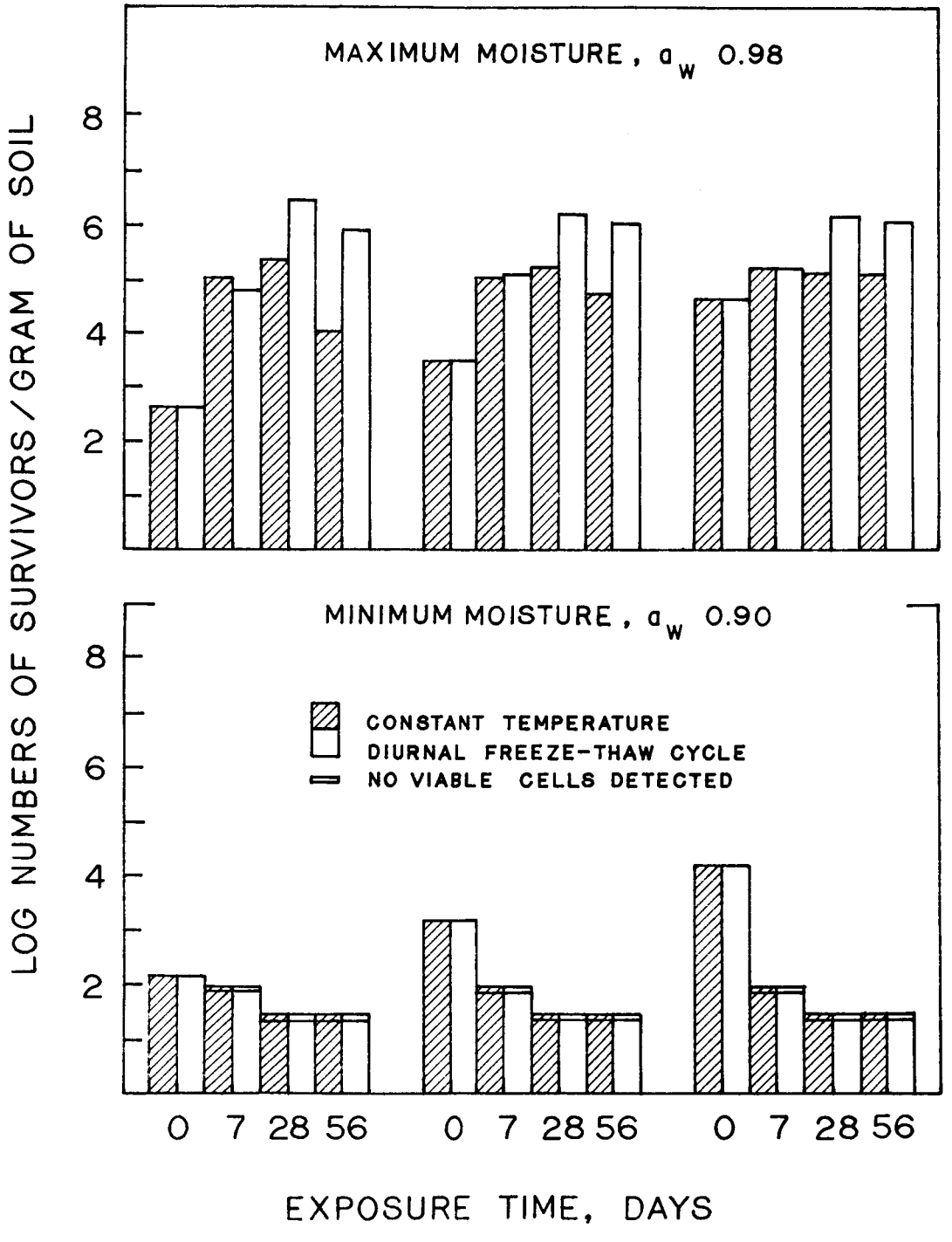

FIG. 9 RELATIONSHIP OF MOISTURE, TEMPERATURE, AND INITIAL CELL NUMBERS OF STAPHYLOCOCCUS AUREUS IN PODZOLIC SOIL.

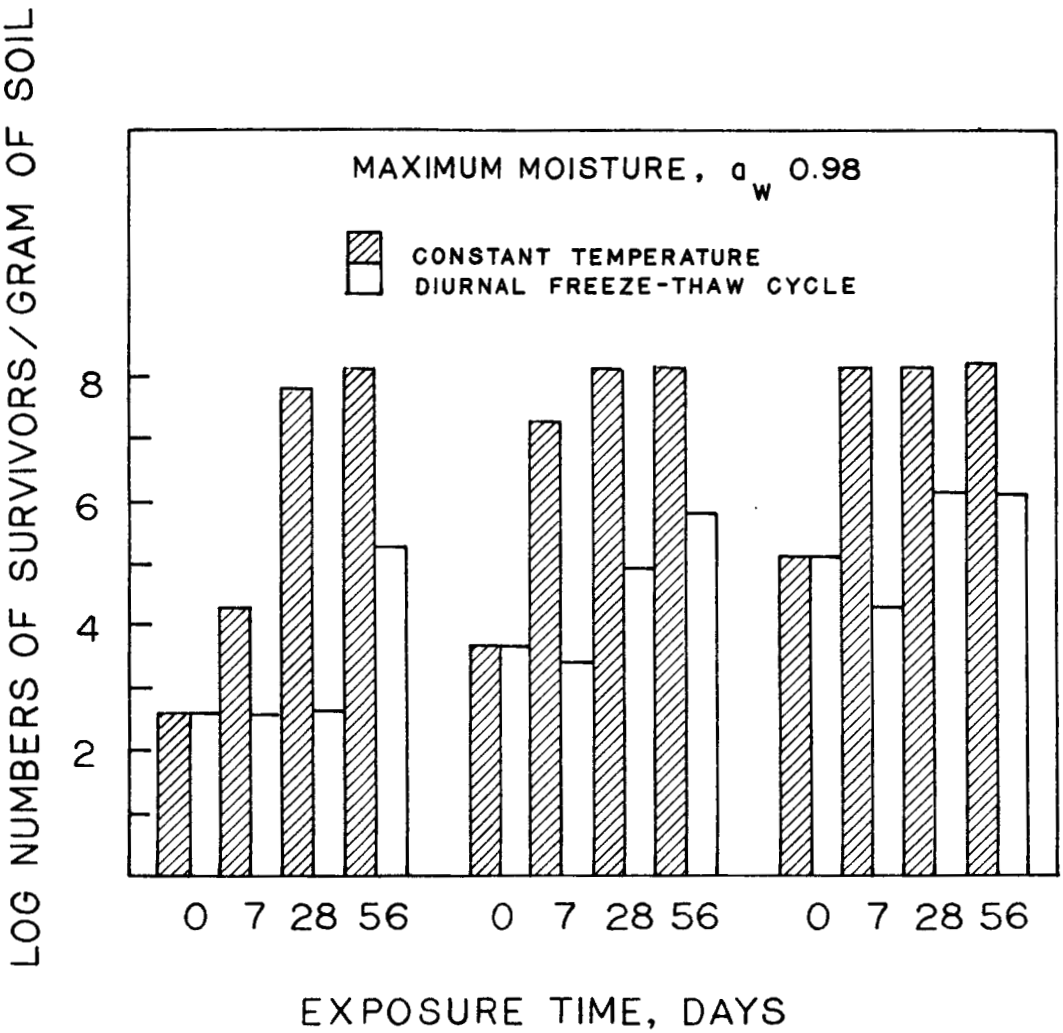

FIG. 10 RELATIONSHP OF MOISTURE, TEMPERATURE, AND INITIAL CELL NUMBERS OF STREPTOMYCES ALBUS IN PODZOLIC SOIL.

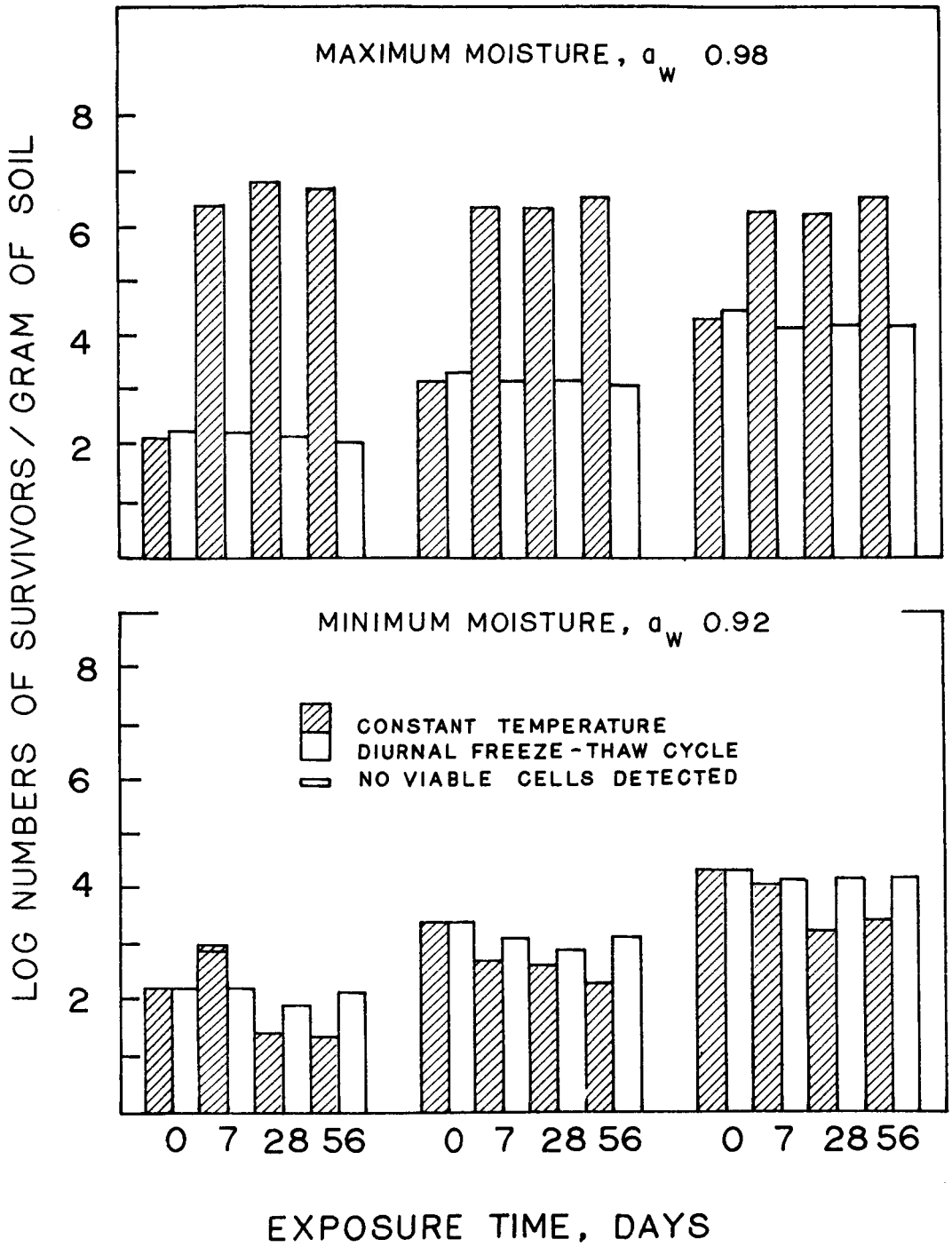

FIG. 11 RELATIONSHIP OF MOISTURE, TEMPERATURE, AND INITIAL CELL NUMBERS OF BACILLUS CEREUS IN PODZOLIC SOIL.

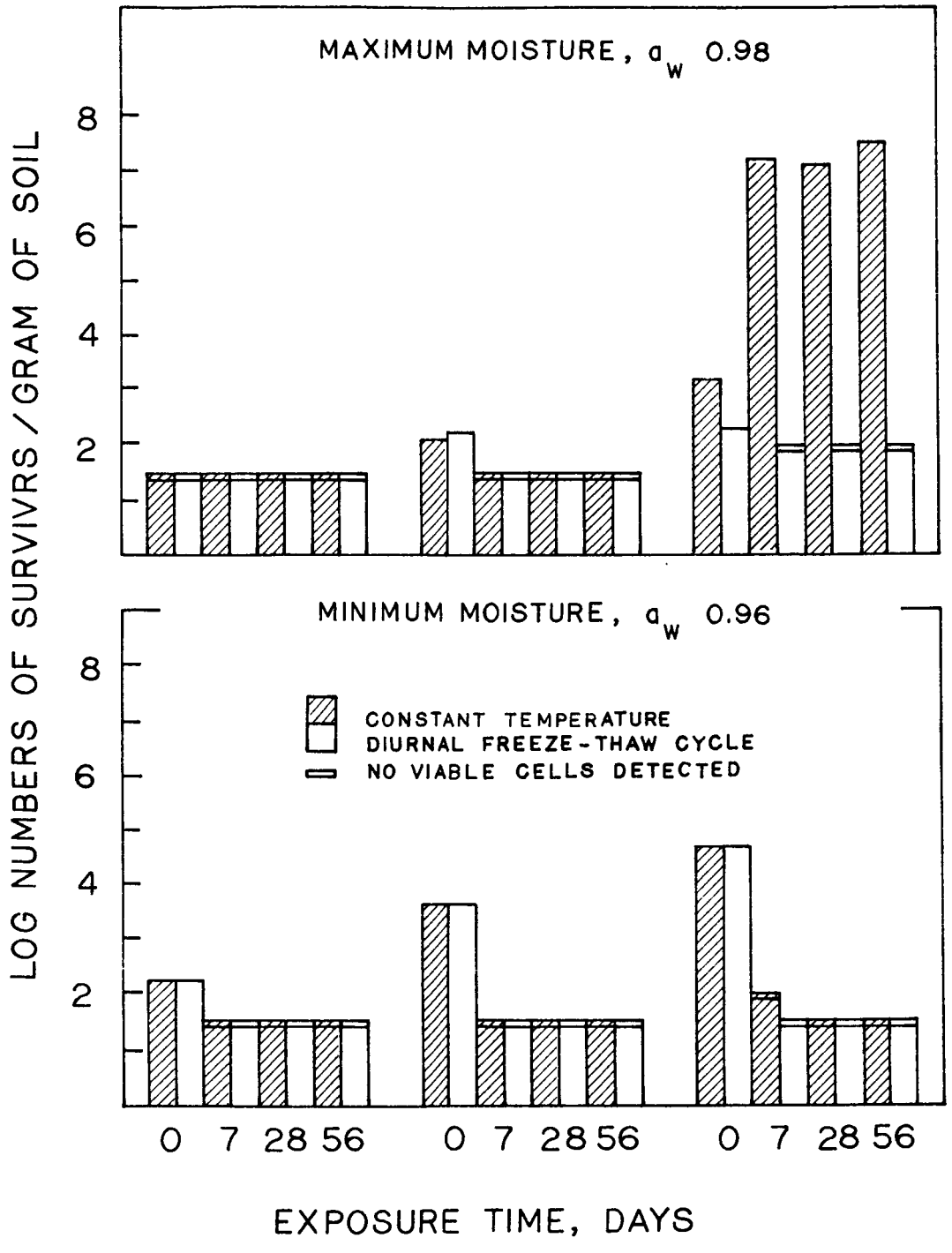

FIG. 12 RELATIONSHIP OF MOISTURE, TEMPERATURE, AND INITIAL CELL NUMBERS OF PSEUDOMONAS AERUGINOSA IN PODZOLIC SOIL.

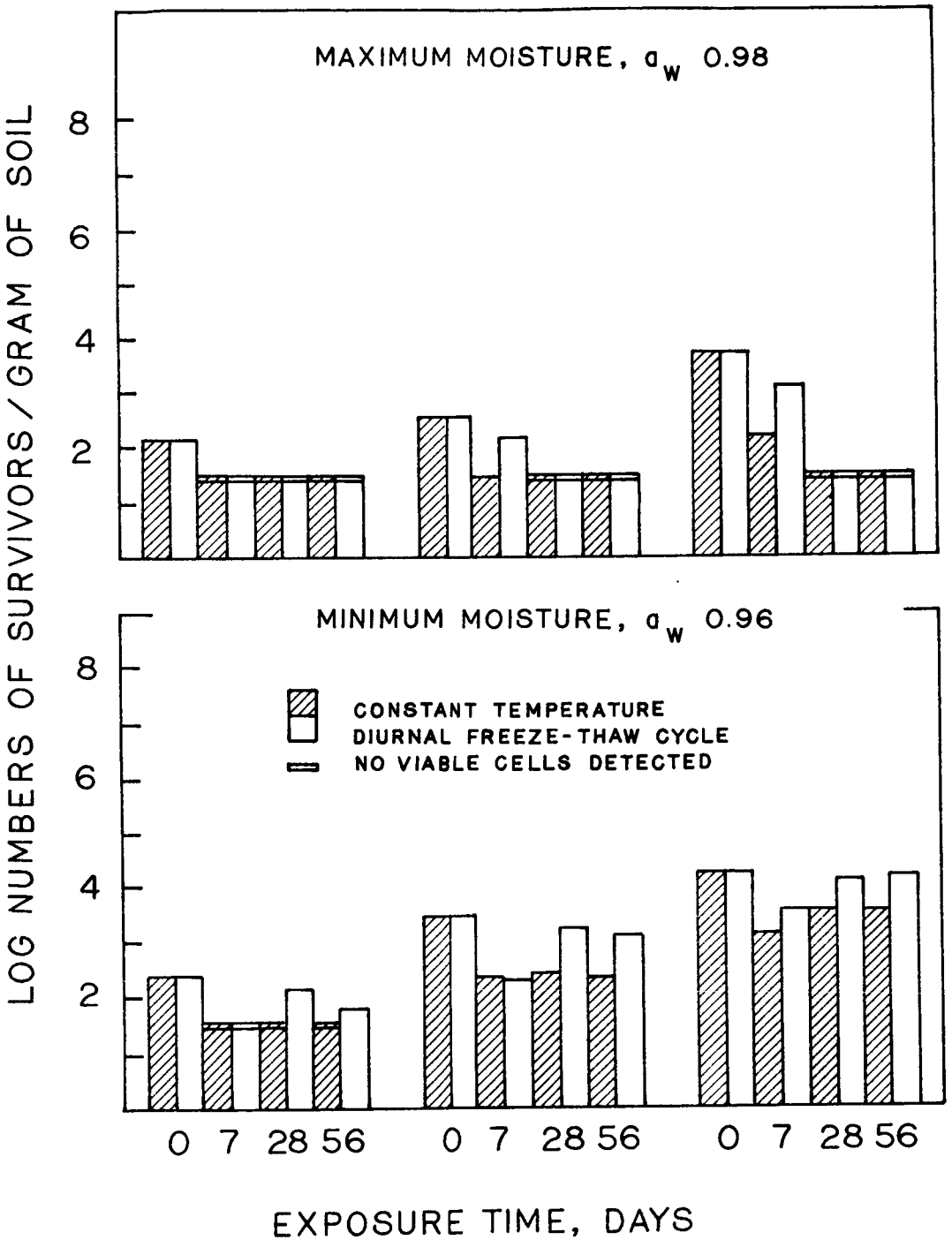

FIG. 13 RELATIONSHIP OF MOISTURE, TEMPERATURE, AND INITIAL CELL NUMBERS OF PUTREFACTIVE ANAEROBE 3679 IN PODZOLIC SOIL.

occurred at the 5×10^2 and 5×10^3 cells/g inoculum level but not with 5×10^4 cells/g.

B. cereus and PA 3679 generally remained stationary regardless of inoculum concentrations or temperature conditions, Figures 11 and 13. S. aureus and P. aeruginosa did not survive 56 days with inocula as high as 10^4 cells/g of soil, Figures 9 and 12.

S. albus growth response was not determined at the minimun a_w level because of the decrease in viability of the stock culture and the difficulty with spore production in various media.

E. Growth Response of Bacteria in Desert Soil

Growth responses of the organisms in desert soil are shown in Figures 14-19. This soil had a very high clay content (48.8%) and with the concentration of the exchangeable cation sodium (106.25 me/100 g of soil) and soluble salt conductivity (100 mmhols/cm) was classified as a saline-alkali type soil. The pH was 8.4 and the organic content 0.27%. Because of these factors the soil served as a very poor substrate for microbial growth or survival.

This group of experiments were only partially successful because maximum a_w levels of 0.98 or 0.99 could not be achieved. The high clay and salt contents effectively bound water so that the maximum a_w was 0.92, a value below minimum requirement for PA 3679, L. plantarum, P. aeruginosa, and S. albus.

B. cereus and PA 3679 survived 56 days in desert soil regardless of the environmental conditions. The survival of these organisms was probably more the result of the spores not germinating than any other factor, Figures 14 and 15.

S. albus inocula of 5×10^4 and 5×10^5 cells/g of soil survived 56 days with a DFT cycle but not with the CT, Figure 16. Inoculum of 5×10^3 cells/g did not survive either temperature condition.

L. plantarum, S. aureus, and P. aeruginosa did not survive in the desert soil with inoculum levels from 5×10^4 to 5×10^5 cells/g of soil, Figures 17 to 19.

F. Overall Growth Responses of Bacteria in the Three Soils

Table 4 summarizes growth responses of the different bacteria in the three types of soil. The brunizemic soil was the best substrate for growth and survival of the selected bacterial species. A greater number of species were able to grow in this soil at all a_w levels and temperature conditions. Fewer species died off completely in this soil than in either the desertic or podzolic soils.

The species best able to grow in the podzolic soil were L. plantarum, S. aureus, and S. albus but growth occurred only at maximum a_w with both CT and DFT cycle.

The desertic soil was the poorest substrate. Only B. cereus and PA 3679 survived and was thought to be from the spores not germinating in the environment.

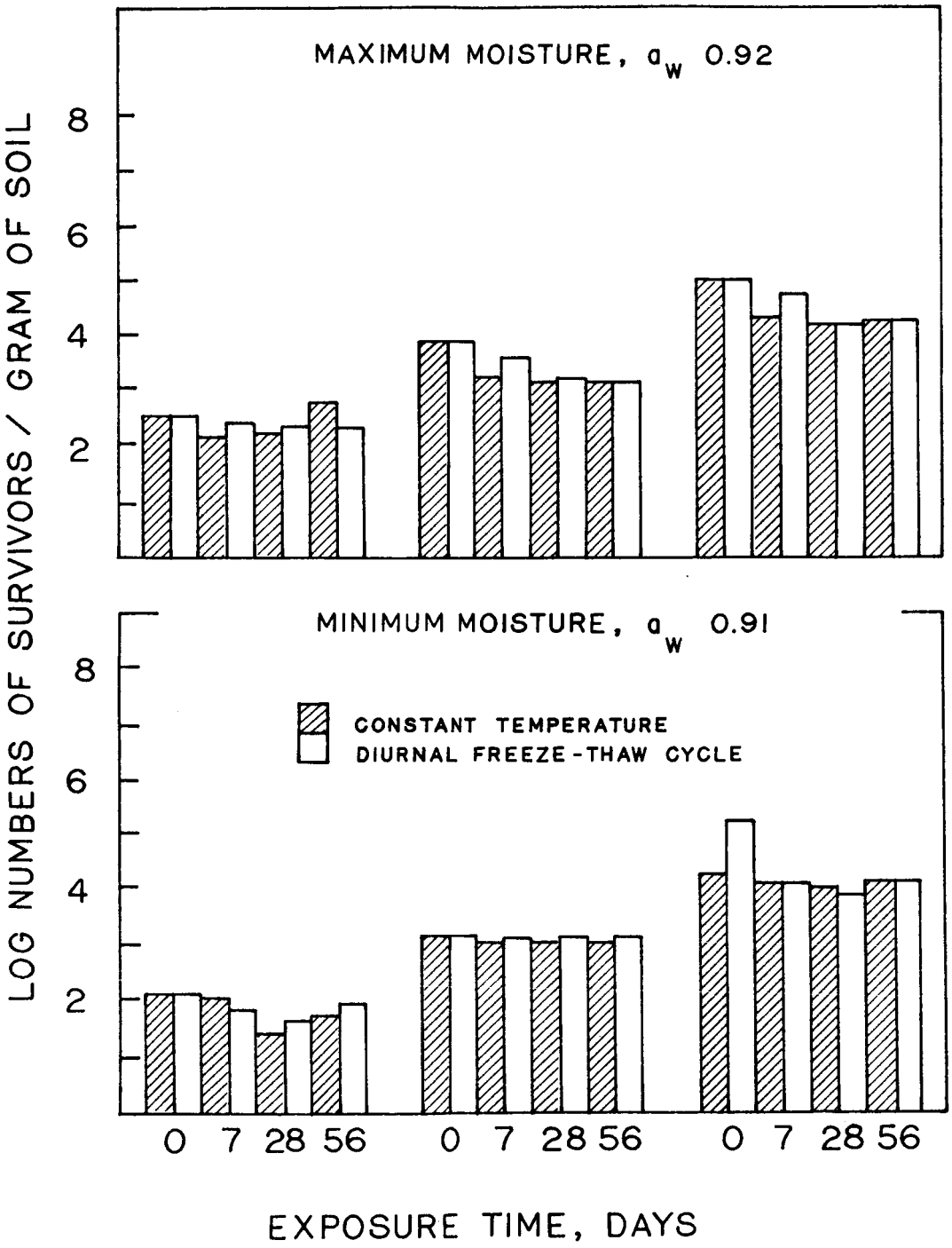

FIG. 14 RELATIONSHIP OF MOISTURE, TEMPERATURE, AND INITIAL CELL NUMBERS OF BACILLUS CEREUS IN DESERTIC SOIL.

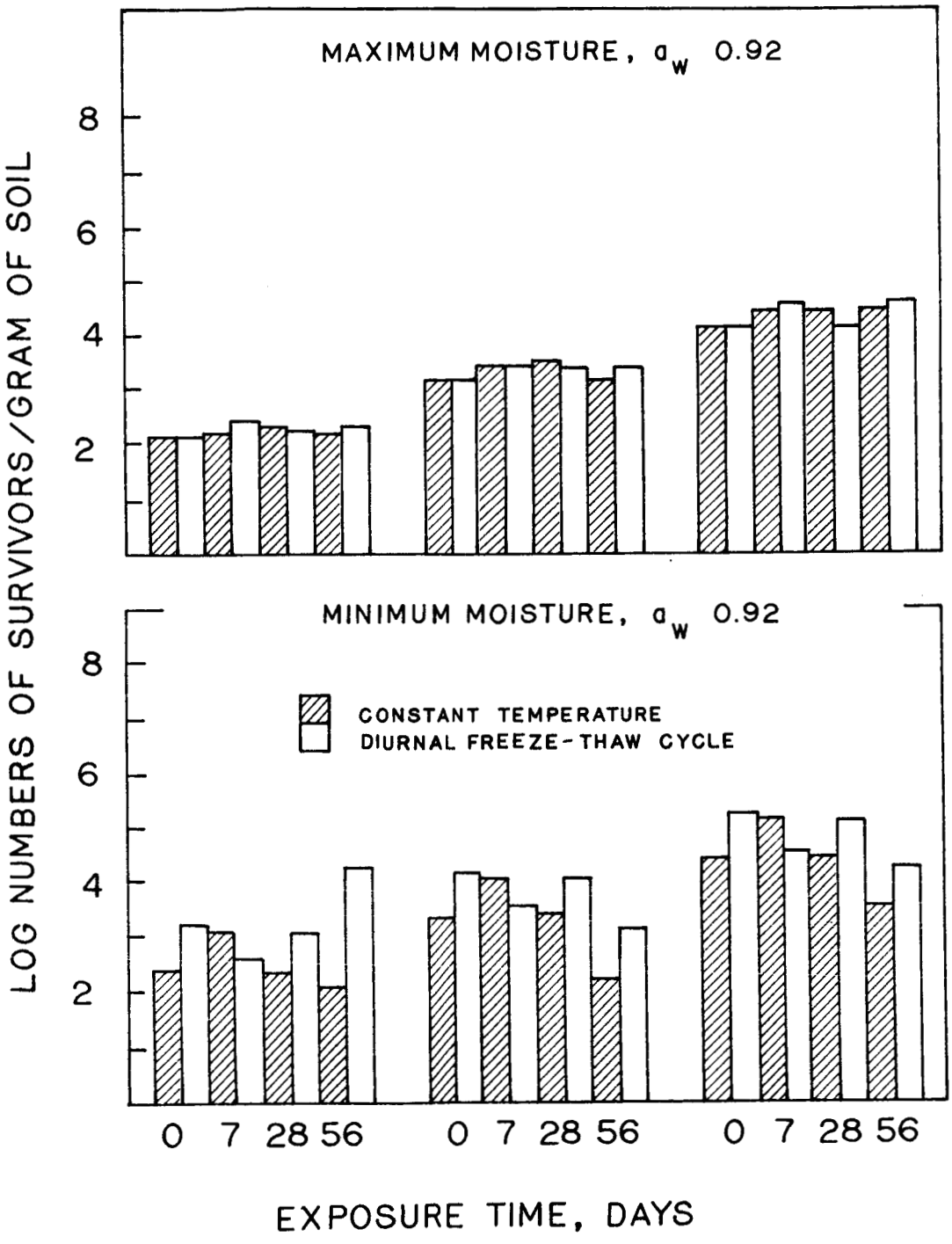

FIG. 15 RELATIONSHIP OF MOISTURE, TEMPERATURE, AND INITIAL CELL NUMBERS OF PUTREFACTIVE ANAEROBE 3679 IN DESERTIC SOIL.

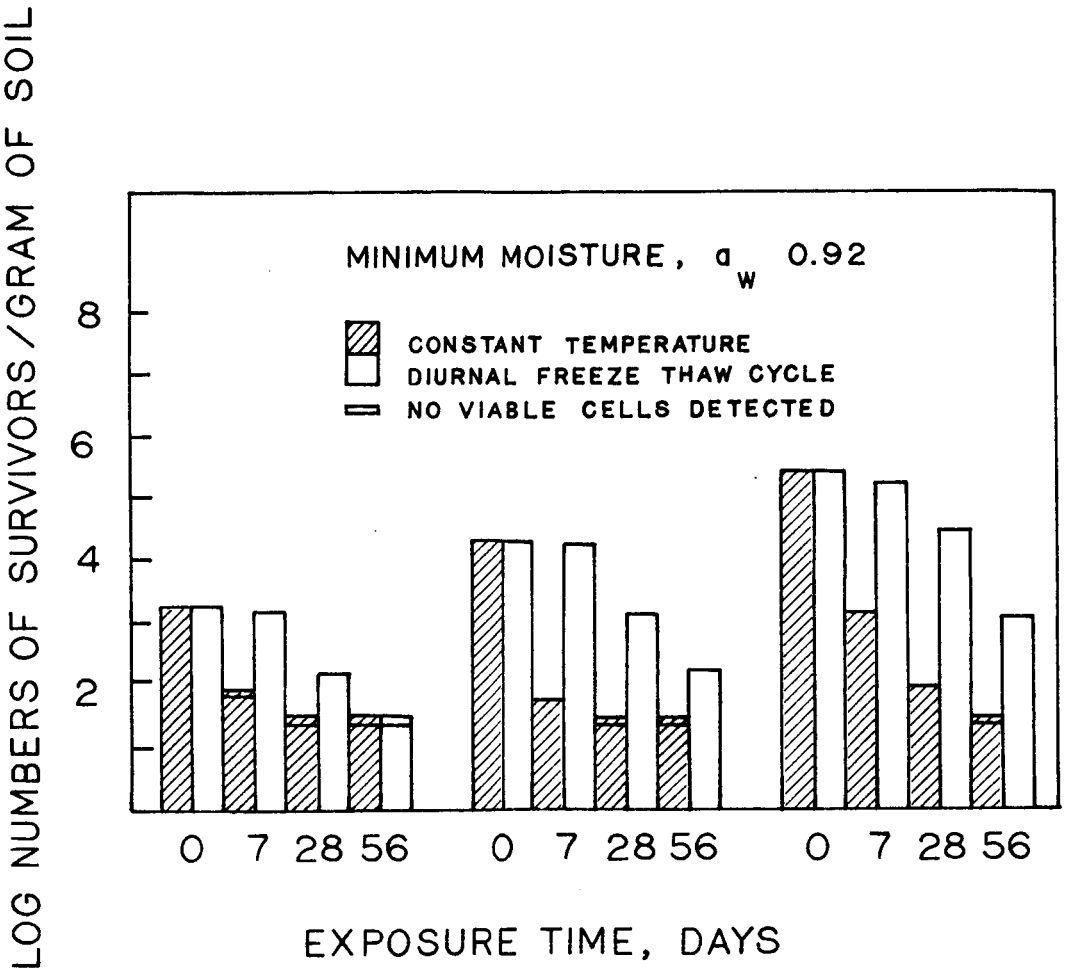

FIG. 16 RELATIONSHIP OF MOISTURE, TEMPERATURE, AND INITIAL CELL NUMBERS OF STREPTOMYCES ALBUS IN DESERTIC SOIL.

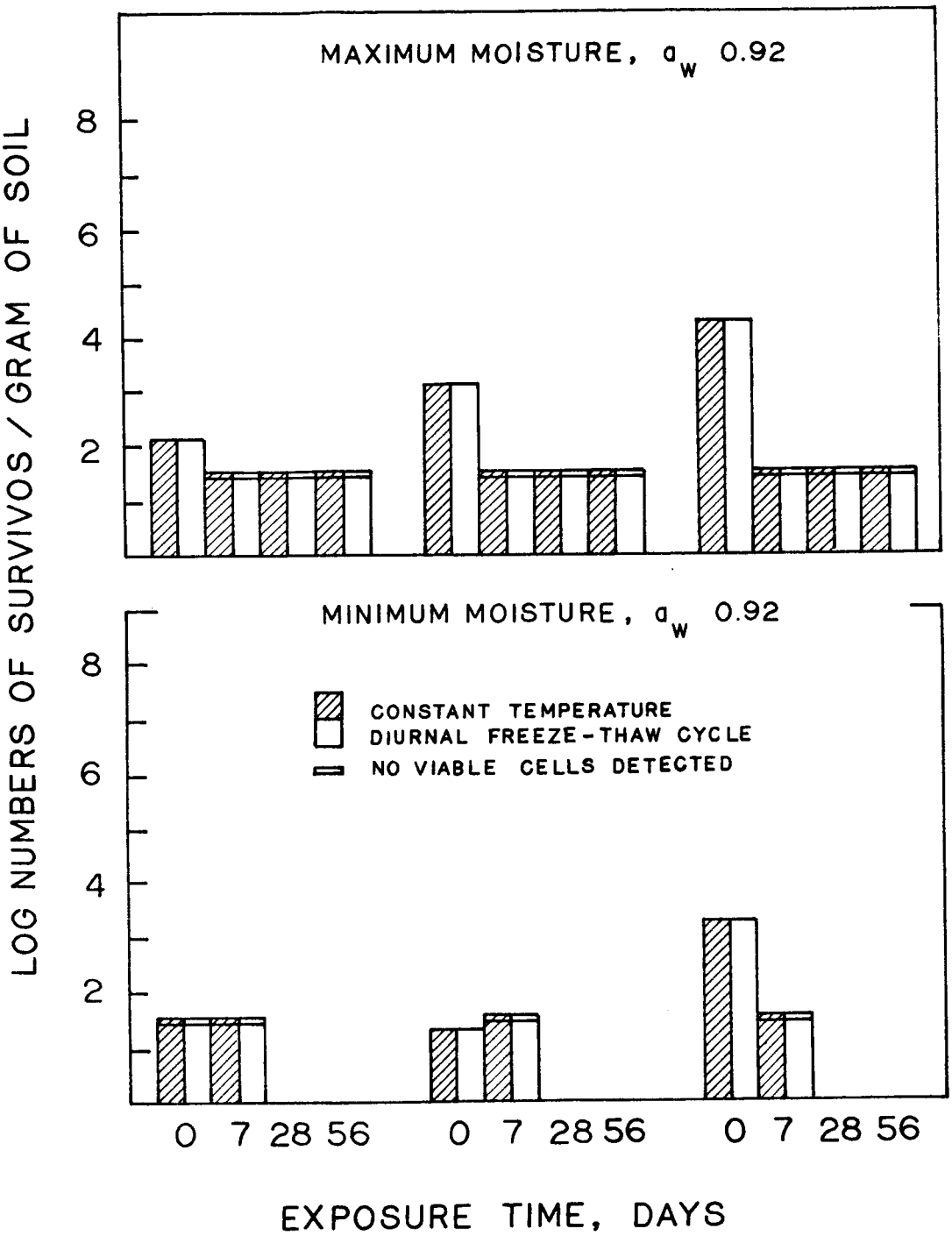

FIG. 17 RELATIONSHIP OF MOISTURE, TEMPERATURE, AND INITIAL CELL NUMBERS OF LACTOBACILLUS PLANTARUM IN DESERTIC SOIL.

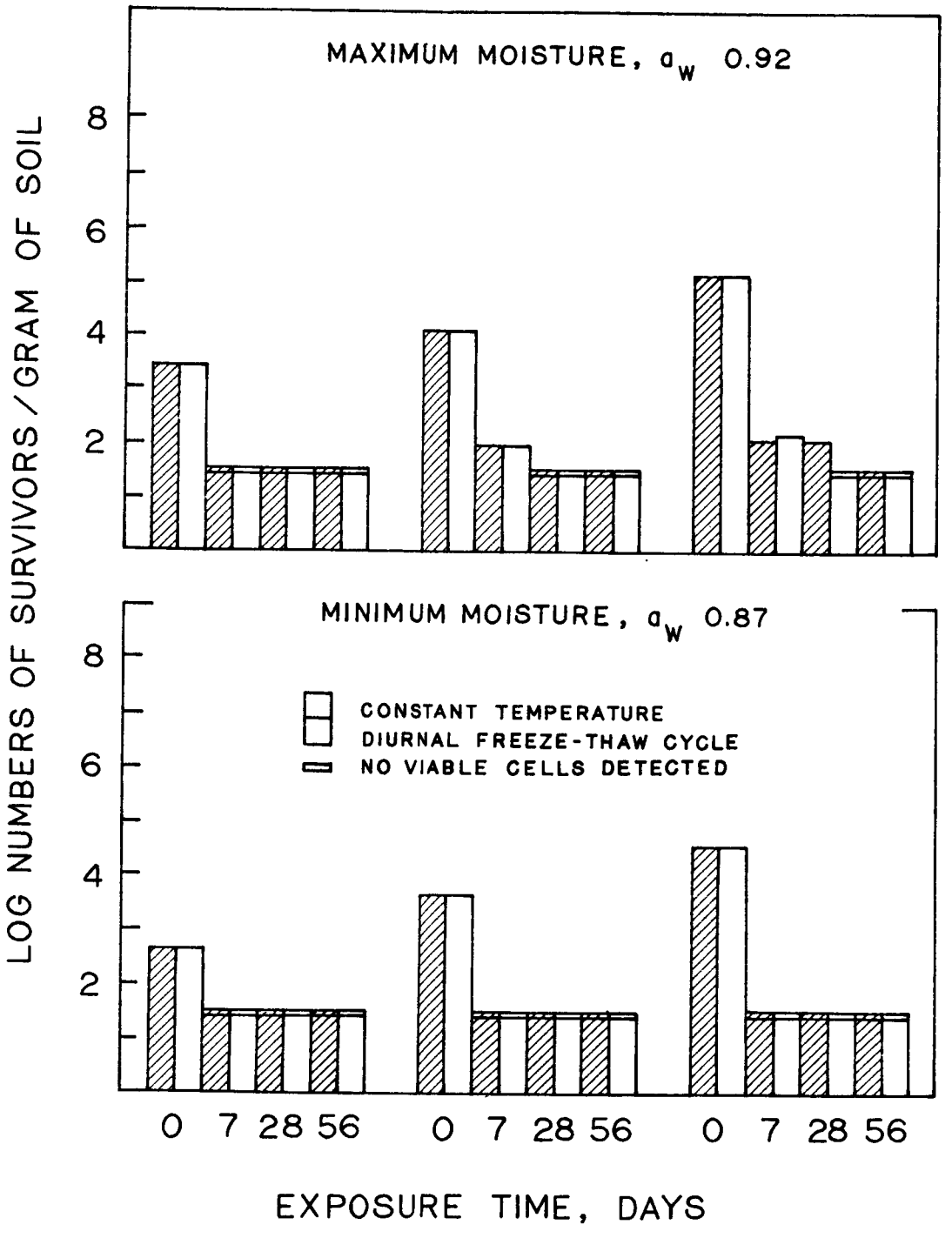

FIG. 18 RELATIONSHIP OF MOISTURE, TEMPERATURE, AND INITIAL CELL NUMBERS OF STAPHYLOCOCCUS AUREUS IN DESERTIC SOIL.

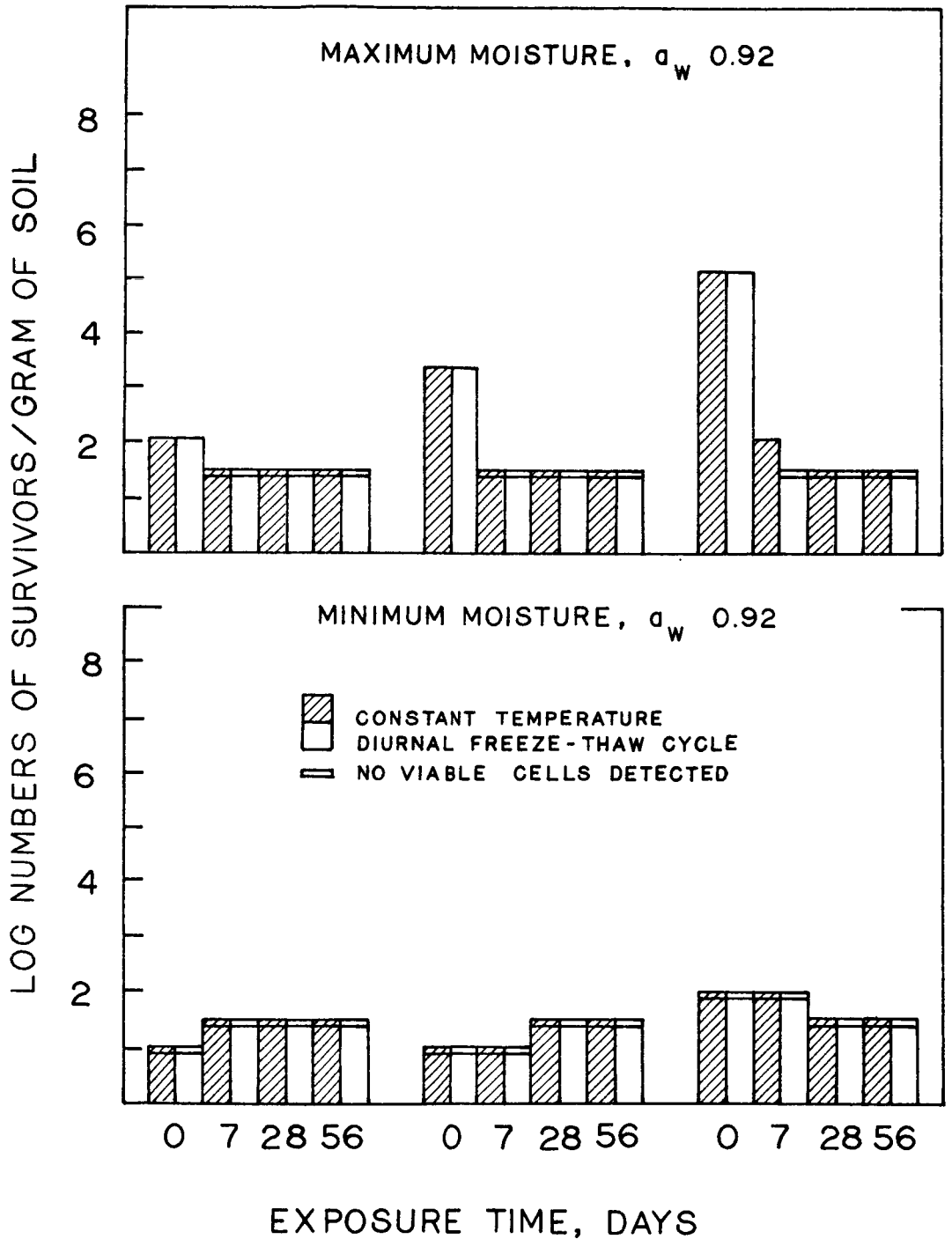

FIG. 19 RELATIONSHIP OF MOISTURE, TEMPERATURE, AND INITIAL CELL NUMBERS OF PSEUDOMONAS AERUGINOSA IN DESERTIC SOIL.

Table 4

EFFECT OF SOIL TYPE ON GROWTH RESPONSE OF BACTERIA

Organism	Number of Cells Inoculated/g of Soil	Ability to Establish an Ecological Niche[a]											
		Brunizemic Soil				Desertic Soil				Podzolic Soil			
		Constant[b]		Diurnal[c]		Constant[b]		Diurnal[c]		Constant[b]		Diurnal[c]	
		Max[d]	Min[e]	Max[d]	Min[e]	Max[d]	Min[e]	Max[d]	Min[e]	Max[d]	Min[e]	Max[d]	Min[e]
B. cereus	10^4	+	+	+	+	-	0	-	0	+	-	0	0
	10^3	+	+	+	+	-	0	-	0	+	-	0	0
	10^2	+	+	+	+	0	0	0	0	+	-	0	0
PA 3679	10^4	-	-	-*	-	0	-	+	0	-*	-	-*	0
	10^3	-*	-	-*	-	0	-	0	0	-*	-	-*	-
	10^2	0	0	0	-	0	-	0	0	-*	-*	-*	-
L. plantarum	10^5	0	-*	+	+	-*	-*	-*	-*	0	-*	+	0
	10^4	+	-*	+	+	-*	-*	-*	-*	+	-*	+	-
	10^3	+	-*	+	+	-*	-*	-*	-*	+	-*	+	+
P. aeruginosa	10^4	+	+	+	+	-*	-*	-*	-*	+	-*	-*	-*
	10^3	+	+	+	+	-*	-*	-*	-*	-*	-*	-*	-*
	10^2	+	+	-*	+	-*	-*	-*	-*	-*	-*	-*	-*
S. aureus	10^5	+	-	+	+	-*	-*	-*	-*	+	-*	+	-*
	10^4	+	-*	+	+	-*	-*	-*	-*	+	-*	+	-*
	10^3	+	-	+	-	-*	-*	-*	-*	+	-*	+	-*
S. albus	10^5	+	+	0	+		-*		-*	+		+	
	10^4	+	+	+	-		-*		-*	+		+	
	10^3	+	+	+	-*		-*		-*	+		+	

[a] + indicates increase, - indicates decrease, and 0 indicates no change in viable cells. Plus or minus scores based on a 3-fold increase or decrease in 56 day counts compared to initial counts.

[b] Incubation at constant 35°C.

[c] Incubation with diurnal 8-hr freeze (-65 C) and 16-hr thaw (30 C) cycle.

[d] Maximum water requirement for a particular organism added to the tubes.

[e] Minimum water requirement for a particular organism added to the tubes.

* Signifies that no viable cells were recovered at the end of 56 days.

Table 5 quantitates the growth responses of the bacteria in the different soils with the different temperatures and a_w conditions.

B. cereus had the best growth response in brunizemic soil of the organisms studied. Inocula of 10^2 cells/g of soil were sufficient for this organism to establish itself with different temperatures and a_w conditions. PA 3679 responded the poorest. Inocula of 10^4 cells/g were sufficient only for survival of this organism.

L. plantarum had the best growth response in the podzolic soil of the organisms studied. Inoculum of 10^2 cells/g of soil were sufficient for growth of this organism with maximum and minimum a_w levels in CT and minimum a_w level in DFT.

P. aeruginosa growth response in podzolic soil was poorest of all the organisms. Inoculum of 10^5 cells/g did not survive minimum a_w levels with CT or DFT cycle, and 10^2 cells/g did not survive maximum a_w with DFT cycle. However, the organism did grow with maximum a_w and CT with 10^3 cells/g of soil inoculum.

In general, population maxima of the organisms that grew in the brunizemic and podzolic soils were 3 to 6 logs higher than initial cell counts which were as low as 10^2 to 10^3 cells/g of soil. In terms of ability to establish an ecological niche as a function of amount of inoculum, moisture concentration, soil type, and temperature condition L. plantarum was the best followed by S. albus, S. aureus, B. cereus, P. aeruginosa, and PA 3679.

Table 5

QUANTITATION OF THE GROWTH RESPONSES OF BACTERIA IN BRUNIZEM, PODZOL, AND DESERT SOILS

Organism	Brunizemic Soil				Podzolic Soil				Desert Soil			
	Constant[1]		Diurnal[2]		Constant[1]		Diurnal[2]		Constant[1]		Diurnal[2]	
	Max[3]	Min[4]	Max[3]	Min[4]	Max[3]	Min[4]	Max[3]	Min[4]	Max[3]	Min[4]	Max[3]	Min[4]
B. cereus	10^2/G[5]	10^2/G	10^2/G	10^2/G	10^2/G	10^2/S	10^2/S	10^2/S	10^3/S	10^2/S	10^3/S	10^2/S
PA 3679	10^4/S	10^4/S	10^4/D	10^4/S	10^4/D	10^4/S	10^4/D	10^2/S	10^2/S	10^2/S	10^2/S	10^3/S
L. plantarum	10^3/G	$<10^2$/G	10^3/G	$<10^2$/G	10^2/G	10^2/G	10^5/D	10^2/G	10^4/D	10^3/D	10^4/D	10^3/D
P. aeruginosa	10^2/G	10^4/G	10^2/G	10^4/G	10^3/G	10^5/D	10^2/D	10^5/D	10^5/D	10^2/D	10^5/D	10^2/D
S. aureus	10^3/G	10^4/S	10^3/G	10^4/G	10^3/G	10^4/D	10^3/G	10^4/D	10^5/D	10^5/D	10^5/D	10^5/D
S. albus	10^3/G	10^3/G	10^2/G	10^4/S	10^3/G	-	10^3/G	-	-	10^5/D	-	10^5/D

[1] Incubation at constant 35°C.

[2] Incubation with diurnal 8-hr freeze (-65°C and 16-hr thaw (30°C) cycle.

[3] Maximum water requirement for a particular organism added to the tubes.

[4] Minimum water requirement for a particular organism added to the tubes.

[5] 10^n/G signifies the lowest log number of organisms that grew.
10^n/S signifies the lowest log number of organisms that survived when no growth occurred.
10^n/D signifies the highest log number of organisms that could not survive.

IV. SUMMARY

L. plantarum was most able to establish an ecological niche with the experimental conditions studied followed by *S. albus*, *S. aureus*, *B. cereus*, *P. aeruginosa*, and PA 3679.

The lowest inoculum tested, 10^2 cells/g of soil, of *B. cereus*, *L. plantarum*, *P. aeruginosa*, and *S. albus* increased 3 to 6 logs; and the lowest number of *S. aureus* tested, 7×10^2 cells/g, increased 4 logs.

The daily freeze-thaw cycles enhanced the growth and survival of *S. aureus* and *L. plantarum* in the brunizemic and podzolic soils.

The growth responses of organisms that grew in the podzolic soil were about 1 log lower than their growth in brunizemic soil.

B. cereus, PA 3679, and *S. albus* were the only bacteria that survived 56 days in the desert. The poor response of bacteria in this soil was caused by the alkaline pH, low organic content, high cation capacity, and high clay content.

There were many instances of an organism surviving and growing in the test environment at the lowest cell inoculum used. Experiments are in progress to determine the lowest inoculum permitting survival or survival and growth of a variety of microorganisms. Other test environments are being considered.

V. PERSONNEL AND RECORDS

The following persons are gratefully acknowledged for their assistance during these studies: Dr. R. E. Cameron, Jet Propulsion Laboratory, Pasadena, California for providing the desert soil and classification information; Messrs. K. C. Hinckley and R. Montgomery, U.S.D.A. Soil Conservation Service, Batavia, Illinois for assistance in collecting the brunizemic and podzolic soils and classification information; and Drs. T. R. Peck and E. C. A. Runge, Department of Agronomy, University of Illinois, Urbana, Illinois for performing the chemical and physical analyses.

The experiments were planned with the counsel of Dr. E. J. Hawrylewicz and the technical assistance of Mr. Bruce Anderson, Miss Marjorie Ewing and Miss Vivian Tolkacz.

Experimental data are recorded in IITRI Logbooks C16684, C16876, C16889, C16938, C17092, C17094, C17260, C17271, C17272, C17497, and C17593.

Respectfully submitted,

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CAH/cg